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(19) (CA) APPLICATION FOR CANADIAN PATENT (12)

(54) Monoclonal Antibody-Containing Agent

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Abstract of the Disclosure

The agent of the present invention comprising a monoclonal antibody which is specifically linked to a protein encoded by human chromosome 21 and which is linked to a protein whose expression in brain varies dependent upon age or a protein which is expressed in only specific cells of brain can be obtained as follows.

Mice are immunized with CHO cells containing long arms of human chromosome 21 (2Fur cells), then the resulting antibody-producing cells are fused with mouse myeloma cells and hybridomas which produce monoclonal antibodies capable of specifically recognizing a protein encoded by human chromosome 21 are selected. A hybridoma which produces a monoclonal antibody capable of recognizing a protein expressed in the human brain tissues is selected from the resulting hybridomas and then the hybridoma is proliferated to give the intended monoclonal antibody.

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MONOCLONAL ANTIBODY-CONTAINING AGENT

Background of the Invention

The present invention relates to an agent which comprises a monoclonal antibody which can recognize a protein encoded by chromosome 21 and expressed in the human brain, which is linked thereto and which is effective for evaluating the function of the human brain and the degree of aging thereof.

Recently, cranial nerve system disorders represented by aphrenesia is becoming a socially and medically important problem as an abrupt increase of the population in the aged persons. For this reason, there has been desired to elucidate the changes of brain functions associated with the aging phenomenon. However, the brain is one of the greatest black boxes in the living body. The human brain has been studied in detail while making use of anatomical and histological means. As a result, the morphological knowledges about the brain have almost been elucidated. There has presently been focused on the elucidation of the correlation between these morphological knowledges and functions of the brain.

One of the methods for elucidating the correlation between the morphology and functions of the brain is a histochemical or cytochemical approach. This

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approach is a method for detecting, *in situ*, a specific function or property of the brain and the approach can make clear a specific function or property thereof through direct microscopic observation thereof. More specifically, sites of the brain exhibiting a specific function or property are selectively stained so as to differentiate it from others.

There have been used monoclonal antibodies in recent histochemical studies of the brain. The monoclonal antibody is a single protein which can recognize only the specific substance (antigen) and can specifically be linked to the substance. The monoclonal antibody is considered to be an important tool for studying brain functions because of this property. In fact, knowledges of brain functions have rapidly been increased due to the histochemical studies which make use of monoclonal antibodies. For instance, only the cholinergic nerve which synthesizes acetylcholine can selectively be stained by reacting a monoclonal antibody to choline acetyltransferase serving as a primary antibody with a section of the brain tissue, then combining it with an enzyme-labelled secondary antibody and subsequently reacting the product with the substrate for the enzyme.

Thus, monoclonal antibodies can widely be used for detecting cranial nerve system disorders. It has become clear that chromosome 21 is the causal gene for

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Alzheimer's disease which has become of interest lately. In addition, the Down's syndrome is a disease in which the nerve cells grow abnormally and the aging of the brain is accelerated. This Down's syndrome is also caused due to a genetic abnormality called trisomy wherein chromosome 21 is three chromosomes. This probably indicates that some of proteins encoded by genes present on chromosome 21 would be involved in functions and growth of nerve systems. For this reason, there have long been required for the development of agents which can recognize proteins encoded by this chromosome 21 and changes in the expression of brain protein due to aging and aphrenesia.

Summary of the Invention

Accordingly, an object of the present invention is to provide an agent comprising a monoclonal antibody which can recognize proteins which are coded by human chromosome 21 and more particularly can recognize a protein whose expression in the human brain varies depending on the age or a protein which is expressed only in a specific cell of the brain.

The agent which permits the achievement of the foregoing object according to the present invention comprises a monoclonal antibody which is specifically linked with a protein encoded by the human chromosome 21

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and large pyramidal cells of the human brain tissue. The monoclonal antibody included in the agent according to the present invention can specifically linked with the cell nucleus protein which has a molecular weight of 32 kilodalton (kDa) and whose quantity of expression in the human brain varies depending on the age.

The monoclonal antibody-containing agent of the present invention can be used as an important diagnostic agent for histochemical studies which is effective in detecting cranial nerve system disorders due to abnormalities in chromosome 21.

Further, the amino acid sequence of the protein which is recognized by the monoclonal antibody included in the agent can be used in the screening of cDNA library (a series of complementary DNA's obtained by reverse transcription of mRNA) and is effective for the elucidation of genes which are expressed and function in the brain.

Brief Explanation of the Drawings

Fig. 1 is a diagram for showing the confirmation of the presence of a protein which can be linked with YO-1 in immunoblot analysis;

Fig 2 is a diagram showing sites in a cell at which a specific protein is localized, the protein being

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linked with YO-1 in immunoblot analysis;

Fig. 3 is a diagram for showing the confirmation of the presence of a protein which can be linked with YO-3 in immunoblot analysis; and

Fig. 4 is a diagram for confirming the fact that YO-1 and YO-3 can recognize the YO-1-linked protein in immunoblot analysis.

In these figures, each reference numeral is as follows: reference numeral 1 represents the analysis of 2Fur cell with YO-1; 2 the analysis of CHO cell with YO-1; 3 the analysis of a second cell nucleus extract of the 2Fur cell with YO-1; 4 the analysis of a first cell nucleus extract of the 2Fur cell with YO-1; 5 the analysis of S-100 cf the 2Fur cell with YO-1; 6 the analysis of the 2Fur cell with YO-3; 7 the analysis of the CHO cell with YO-3; 8 the analysis of an YO-1-linked protein with YO-1; and 9 the analysis of the YO-1-linked protein with YO-3.

Detailed Explanation of the Invention

The monoclonal antibody included in the agent of the present invention can be prepared in the following manner.

Mice are immunized with 2Fur cells (CHO cells containing long arms of the human chromosome 21) to give

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antibody-producing cells, the antibody-producing cells are fused with mouse myeloma cells and then hybridomas which can produce the monoclonal antibodies capable of specifically recognizing a protein encoded by the human chromosome 21. A hybridoma which can produce a monoclonal antibody capable of recognizing a protein expressed in the cells of the human brain is then isolated from the resulting hybridomas. The hybridoma can be proliferated to give the foregoing monoclonal antibody.

Examples of the present invention will hereinafter be described but the present invention should not be limited by these examples.

Example 1

Preparation of Monoclonal Antibody YO-1

2Fur cells (10^6 cells; CHO cells including long arms of the human chromosome 21; D. Patterson et al., Som. Cell Genet., 1975, 1, p.91) were intraperitoneally injected into BALB/c mice every two weeks over three times. One week after the injection, the mice were subjected to booster in the same manner. Three days after the booster, the spleens were removed from the mice, suspended in a DF medium (containing, in 1,000 ml of the medium, 5.25 g of Dulbecco-Modified Eagle (DME) medium, 5.57 g of Ham F12 (F12), 3.57 g of HEPES (2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 0.1 g of Streptomycin, 10⁵ units of Penicillin and

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1.4 g of NaHCO₃), mixed with myeloma cells (P3/X63-Ag8.U1) in a ratio of 10:1 and centrifuged at 1,600 rpm for 5 minutes. After adding 50% polyethylene glycol and gently stirring the resulting mixture, the cells were washed through centrifugation (1,000 rpm, 10 min). Thereafter, the spleen cells and the myeloma cells (10⁶ cells/ml) were suspended in a DF medium containing 15% fetal calf serum. 0.1 ml thereof was dispensed in a plate provided with 96 wells and cultured in a CO₂-cultivation apparatus for one day. Thereafter, these cells were cultured in a HAT medium (a medium obtained by dissolving 1.36 mg of hypoxanthine, 0.39 mg of thymidine and 0.0182 mg of aminopterin in 100 ml of the DF medium).

The selection of hybridomas was carried out by dissolving 2Fur cells of CHO cells in a lysing buffer (a solution containing 0.5844 g of NaCl, 0.4 ml of NP40 (NONIDET P-40; available from Sigma Company) and 0.5 ml of aprotinin in 100 ml of a 10 mM Tris-HCl buffer (pH 8.0)), absorbing the resulting solution onto a plate provided with 96 wells, subjecting the culture supernatant to enzyme-linked immunosorbent assay (ELISA) with respect to the resulting plate and finally selecting a 2Fur-positive and CHO-negative clone.

The resulting clone was proliferated in the peritoneal cavity of BALB/c mice and the resulting ascites were used as a crude monoclonal antibody solution. To

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confirm whether the monoclonal antibody thus obtained could specifically recognize the protein encoded by the human chromosome 21, the 2Pur cells and the CHO cells were subjected to immunoblotting in the following manner. The 2Pur cells or the CHO cells were dissolved in the same lysing buffer and then the resulting solution was subjected to SDS (sodium dodecyl sulfate)-polyacrylamide gel electrophoresis according to the method of Laemmli (see Nature, 1970, 227, p. 680). After the electrophoresis, the SDS was removed from the gel and the protein was transferred to a nitrocellulose film by applying an electric current (a constant current of 150 mA) for 2 hours using a transfer apparatus (available from Toyo Company). After the transfer, blocking was carried out using a 5% skim milk solution. The nitrocellulose film was washed with TBS (50 mM Tris-HCl, 200mM NaCl, pH 7.4) containing 0.05% Tween 20 and then reacted in the crude monoclonal antibody solution diluted 1,000 times with TBS at 37 °C for 2 hours. After washing, the reaction product was reacted with alkaliphosphatase labelled anti-mouse IgG (Tago) at 37 °C for 2 hours. Nitroblue tetraazolium and 5-bromo-4-chloro-3-indolyl phosphate were used for the coloration.

Results

As shown in Fig. 1, there was obtained monoclonal

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antibody YO-1 (PERM SP-3123; hereinafter referred to as "YO-1") capable of specifically recognizing the protein having a molecular weight of 32 KDa derived from the 2Fur cell. This protein is not expressed in the CHO cell. This fact clearly indicates that the foregoing protein is encoded by a gene present on the human chromosome 21.

Expression of YO-1-linked Protein in Brain

In the preparation of specimens of brain tissues, a frozen section was prepared by cutting frozen brain tissue into pieces having a thickness ranging from 10 to 20 μ m with a cryostat and then adhered to a slide glass on which egg albumin had been coated and, on the other hand, the slide glass on which paraffin is mounted was immersed twice in xylene for 10 minutes for the removal of the paraffin and then treated with ethanol and distilled water for the removal of the xylene.

After reacting the section of the brain tissue adhered to the slide glass with YO-1, the tissue was stained according to the avidinbiotin complex method (ABC method) or peroxidase-antiperoxidase method (PAP method). Then the section was subjected to the counter staining with hematoxylin, treated with ethanol and xylene and then sealed with canada balsam. These specimens were examined by a light microscope.

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Results

The YO-1-linked protein was expressed in large pyramidal neuron of nerve cells in the brain tissue. There was not observed any expression in the granulocytes.

Detection of Proteins Whose Expression is Dependent Upon Age

In the same manner used in the foregoing expression in the brain, brain tissues derived from normal persons and patients suffering from Down's disease, of various ages, were reacted with YO-1, the tissues were stained and examined through microscopic observation.

Results

Table 1 shows the results of YO-1-linked protein expression in the large pyramidal cells of the brain. The YO-1-linked protein expression was positive in all the examined normal persons of 2 years old or older. On the other hand, it was already expressed in the brain of foetus of 40-weeks-old in case of patients suffering from Down's disease. Its expression was positive in both pyramidal cells and granulocytes in case of anti-superoxide dismutase (SOD) monoclonal antibody used as a control. In the latter case, there was not observed any difference between the normal persons and the patients suffering from Down's disease.

Moreover, glia cells in the brain was likewise

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examined through microscopic observation. Table 2 shows the results of the YO-1-linked protein expression in the glia cells. The YO-1-linked protein expression was negative in astrocytes, oligodendrocytes and microglia. On the other hand, if staining was performed with anti-SOD monoclonal antibody as a control, positive glia cells were detected in both normal persons and patients suffering from Down's disease, of 40-week-old (foetus) or older.

Table 1

<u>Death Age</u>	<u>Normal</u>	<u>YO-1</u>	<u>Anti-SOD*</u>	
			<u>Normal</u>	<u>Down's Disease</u>
17 weeks foetus	-	-	-	-
25 weeks foetus	-	-	-	-
40 weeks foetus	-	±	+	++
3 months infant	-	±	+	+
2 years infant	+	+	+	++
32 years adult	±	++	++	++

± : weakly expressed; + : expressed in a part of cells;

++ : expressed ; - : not expressed.

*Anti-SOD: anti-superoxide dismutase.

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Table 2

<u>Death Age</u>	<u>Normal</u>	<u>YO-1</u>	<u>Anti-SOD*</u>
		<u>Down's Disease</u>	<u>Normal Down's Disease</u>
17 weeks foetus	-	-	-
25 weeks foetus	-	-	-
43 weeks foetus	-	-	++ +
3 months infant	-	-	++ +
2 years infant	-	-	++ +
32 years adult	-	-	++ +

± : weakly expressed; + : expressed in a part of cells;

++ : expressed ; - : not expressed.

*Anti-SOD: anti-superoxide dismutase.

Sites at Which YO-1-linked Protein is Localized

A buffer solution A (10 mM HEPES, pH 7.9; 1.5 mM MgCl₂; and 10 mM DTT) was added to 2Fur cells in an amount of 10 ml per 1x10⁶ cells, the resulting mixture was homogenized 10 times with a Teflon homogenizer and then centrifuged at 2,000 rpm for 10 minutes to give a supernatant which was referred to as S-100 fraction. To the precipitates, there was added 30 ml of a buffer solution C (20 mM HEPES, pH 7.9; 25% glycerol; 0.42 M NaCl; 1.5 mM MgCl₂; 0.2 mM EDTA; 0.5 mM PMSF; and 0.5 mM DTT), the resulting mixture was homogenized 10 times with a Teflon homogenizer and then centrifuged at 16,000 rpm for 20 minutes to give a supernatant which was referred to as a first cell nucleus extract. The precipitates were

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further suspended in 40 ml of the same buffer solution C, the resulting mixture was homogenized 10 times with a Teflon homogenizer and then centrifuged at 35,000 rpm for 30 minutes to give a supernatant which was referred to as a second cell nucleus extract. The S-100 fraction and the first and second cell nucleus extracts each was dialyzed against phosphate buffered saline (PBS) at 4 °C overnight. These various protein fractions were separated by SDS-polyacrylamide gel electrophoresis respectively and subjected to immunoblotting in which YO-1 was used as a primary antibody.

Results

The protein having a molecular weight of 32 kDa which was recognized by YO-1 was present in the second cell nucleus extract as shown in Fig. 2. Thus, it was elucidated that YO-1 was linked with the cell nucleus protein.

Purification of YO-1-Linked Protein

YO-1 derived from the mouse ascites (4 mg, 10 ml) which had been purified by Affi-gel protein A (available from Bio-Rad) and dialyzed against PBS at 4 °C for 3 hours was suspended in 0.6 g of swollen CNBr-activated Sepharose 4B (available from Pharmacia), incubated at 4 °C overnight to adsorb YO-1 onto the CNBr-activated Sepharose 4B and

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to thus from YO-1-Sepharose 4B.

The resulting YO-1-Sepharose 4B was packed in a column, washed with PBS and then the second cell nucleus extract was passed through the column at a flow rate of 10ml/hr to adsorb proteins onto the column. Elution was performed using 0.1 M glycine-HCl (pH 2.5). The proteins eluted were neutralized with 2 M Tris-HCl (pH 8.0). The resulting proteins were further purified by reverse phase high performance liquid chromatography (reverse phase HPLC; TSK gel Phenyl-5PW; available from Tosoh Corporation). The proteins were concentrated to dryness by lyophilization, dissolved in 25 mM Tris-HCl (pH 8.5) containing 1 mM EDTA followed by the addition of an endoproteinase Lys-C (available from Behringwerke Manheim) in an amount of 1/10 time that of the proteins and reaction performed at 37 °C overnight. Peptide fragments obtained after the digestion were isolated by TSK gel ODS-120T (available from Tosoh Corporation). Each peptide fragment isolated was concentrated to dryness by a centrifugal evaporator, dissolved in ultrapure water and then the amino acid sequence thereof was determined by a Gas Phase Protein Sequencer (Shimazu PQS-1).

Results

As a result of the reverse phase HPLC separation, it was found that the YO-1-linked protein having a molecular

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weight of 32 KDa was decomposed into about 30 peptide fragments. Peptide fragments showing main 8 peaks had the following amino acid sequences.

No. 12: TPK; No. 21: ATGSATPK;
No. 22: KPAAAATVK; No. 24: GTGASGSFK;
No. 27: LGLK; No. 29: ALAAAGYDVEK;
No. 30: ERSGVSLAALK; No. 31: ASGPPVSELITK.

Each symbol stands for the following amino acid residue (Each No. represents the order of the corresponding peak appeared).

A: Alanine;	C: Cysteine;
D: Aspartic acid;	E: Glutamic acid;
F: Phenylalanine;	G: Glycine;
H: Histidine;	I: Isoleucine;
K: Lysine;	L: Leucine;
M: Methionine;	N: Asparagine;
P: Proline;	O: Glutamine;
R: Arginine;	S: Serine;
T: Threonine;	V: Valine;
W: Tryptophan;	Y: Tyrosine.

Example 2

Preparation of Monoclonal Antibody YO-3

2Fur cells (10^7 cells; CHO cells including long arms of the human chromosome 21; D. Patterson et al., Som. Cell Genet., 1975, 1, p. 91) were intraperitoneally injected

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into BALB/c mice every two weeks over three times. One week after the injection, the mice were subjected to booster in the same manner. Three days after the booster, the spleens were removed from the mice, suspended in a DF medium (containing, in 1,000 ml of the medium, 5.25 g of Dulbecco-Modified Eagle (DME) medium, 5.57 g of HUM F12 (F12), 3.57 g of HEPES (2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 0.1g of Streptomycin, 10⁶ units of Penicillin and 1.4g of NaHCO₃), mixed with myeloma cells (P3/X63-Ag8.U1) in a ratio of 10:1 and centrifuged at 1,600 rpm for 5 minutes. After adding 50% polyethylene glycol and gently stirring the resulting mixture, the cells were washed through centrifugation (1,000 rpm, 10 min). Thereafter, the spleen cells and the myeloma cells (10⁶ cells/ml) were suspended in a DF medium containing 15% fetal calf serum, 0.1 ml thereof was dispensed in a plate provided with 96 wells and cultured in a CO₂-cultivation apparatus for one day. Thereafter, these cells were cultured in a HAT medium (a medium obtained by dissolving 1.36 mg of hypoxanthine, 0.39 mg of thymidine and 0.0182 of aminopterin in 100 ml of the DF medium).

The selection of hybridomas was carried out by dissolving 2Fur cells or CHO cells in a lysing buffer (a solution containing 0.5844 g of NaCl, 0.4 ml of NP40 (NONIDET P-40; available from Sigma Company) and 0.5 ml of aprotinin in 100 ml of a 10 mM Tris-HCl buffer (pH 8.0)),

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adsorbing the resulting solution onto a plate provided with 96 wells, subjecting the culture supernatant to enzyme-linked immunosorbent assay (ELISA) with respect to the resulting plate and finally selecting a 2Fur-positive and CHO-negative clone.

The resulting clone was proliferated in the peritoneal cavity of BALB/c mice and the resulting ascites were used as a crude monoclonal antibody solution. To confirm whether the monoclonal antibody thus obtained could specifically recognized the protein encoded by the human chromosome 21, the 2Fur cells and the CHO cells were subjected to immunoblotting in the following manner. The 2Fur cells or the CHO cells were dissolved in the same lysing buffer and then the resulting solution was subjected to SDS (sodium dodecyl sulfate)-polyacrylamide gel electrophoresis according to the method of Laemmli (see Nature 1970, 227, p. 680). After the electrophoresis, the SDS was removed from the gel and the protein was transferred to a nitrocellulose film by applying an electric current (a constant current of 150 mA) for 2 hours using a transfer apparatus (available from Toyo Company). After the transfer, blocking was carried out using a 5% skimmilk solution. The nitrocellulose film was washed with TBS (50 mM Tris-HCl, 200 mM NaCl, pH 7.4) containing 0.05% Tween 20 and then reacted in the crude monoclonal antibody solution diluted 1,000 times with TBS at 37 °C

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for 2 hours. After washing, the reaction product was reacted with alkaliphosphataselabelled anti-mouse IgG (Tago) at 37° C for 2 hours. Nitroblue tetraazolium and 5-bromo-4-chloro-3-indolyl phosphate were used for the coloration thereof.

Results

As shown in Fig. 3, there was obtained a monoclonal antibody YO-3 (FERM BP-3423; hereinafter referred to as "YO-3") capable of specifically recognizing the proteins having molecular weights of 32 KDa and 23 KDa respectively derived from the 2Fur cell and the protein having a molecular weight of 23 KDa derived form the CHO cells.

Expression of YO-3-linked Protein in Brain

Specimens of brain tissue were prepared as follows. A frozen section was prepared by cutting frozen brain tissue into pieces having a thickness ranging from 10 to 20 μ m with a cryostat and then adhered to a slide glass on which had been coated and, on the other hand, a paraffin section mounted on a slide glass was immersed twice in xylene for 10 minutes to remove the paraffin and then treated with ethanol and distilled water to remove the xylene.

After reacting the section of the brain tissue

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adhered to the slide glass with YO-3, the tissue was stained according to the avidin-biotin complex method (ABC method) or peroxidase-antiperoxidase method (PAP method). Then the section was subjected to the counter staining with hematoxylin, treated with ethanol and xylene and then sealed with canada balsam. These specimens were examined by a light microscope.

Results

The YO-3-linked protein was expressed in large pyramidal neuron of nerve cells in the brain tissue like the YO-1-linked protein. There was not observed any expression in the small granulocytes.

Detection of Proteins Whose Expression is Dependent Upon Age

In the same manner used in the foregoing expression in the brain, brain tissue derived from normal persons and patients suffering from Down's disease, of various ages were reacted with YO-3, the tissues were stained examined through microscopic observation.

Results

Table 3 shows the results of YO-3-linked protein expression in the large pyramidal cells of the brain. The YO-3-linked protein expression was positive in both examined normal persons and patients suffering from

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Down's disease, of 40-week-old or older. There was not observed any expression of the YO-3-linked protein in the glia cells of the brain as in the case of the YO-1-linked protein.

Table 3

<u>Death Age</u>	<u>Normal</u>	<u>Down's Disease</u>	<u>Anti-SOD*</u>	
			<u>YO-3</u>	<u>Anti-SOD*</u>
17 weeks foetus	-	-	-	-
25 weeks foetus	-	-	-	-
40 weeks foetus	+	±	+	++
3 months infant	±	+	+	+
2 years infant	+	++	+	++
32 years adult	++	++	++	++

± : weakly expressed; + : expressed in a part of cells;
++ : expressed ; - : not expressed.

*Anti-SOD: anti-superoxide dismutase.

Homology of YO-1-Linked Protein and YO-3-Linked Protein

YO-1 derived from the mouse ascites (4 mg, 10 ml) which had been purified by Affi-gel protein A (available from Bio-Rad) and dialyzed against PBS at 4 °C for 3 hours was suspended in 0.6 g of swollen CNBr-activated Sepharose 4B (available from Pharmacia), incubated at 4 °C overnight to adsorb YO-1 onto the CNBr-activated Sepharose 4B and to thus form YO-1-Sepharose 4B.

The resulting YO-1-Sepharose 4B was packed in a

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column, washed with PBS and then the second cell nucleus extract was passed through the column at a flow rate of 10 ml/hr to adsorb proteins onto the column. Elution was performed using 0.1 M glycine-HCl (pH 2.5). The proteins eluted were neutralized with 2 M Tris-HCl (pH 8.0). The resulting proteins were further purified by reverse phase high performance liquid chromatography (reverse phase HPLC; TSK gel Phenyl-5PW; available from Tosoh Corporation).

The purified proteins were dissolved in the lysing buffer and then the resulting solution was subjected to SDS (sodium dodecyl sulfate)-polyacrylamide gel electrophoresis according to the method of Laemmli (see Nature, 1970, 227, p. 680). After the electrophoresis, the SDS was removed from the gel and the protein was transferred to a nitrocellulose film by applying an electric current (a constant current of 150 mA) for 2 hours using a transfer apparatus (available from Toyo Company). After the transfer, the nitrocellulose film was blocked with a 5% skimmed milk solution. The nitrocellulose film was washed with TBS (50 mM Tris-HCl, 200 mM NaCl, pH 7.4) containing 0.05% Tween 20 and then reacted in a solution containing YO-3 diluted with TBS at 37 °C for 2 hours. After washing, the reaction product was reacted with alkaliphosphatase labeled anti-mouse IgG (Tago) at 37 °C for 2 hours. Nitroblue tetraazolium and 5-bromo-4-chloro-3-indolyl phosphate were used for the coloration thereof.

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By way of comparison, immunoblotting in which YO-1 was used as a primary antibody was also carried out.

Results

As shown in Fig. 4, YO-3 could recognize the YO-1-linked protein. Thus, it is confirmed that the YO-1-linked protein of 32 KDa specific to the 2Fur cell is identical to the YO-3-linked protein.

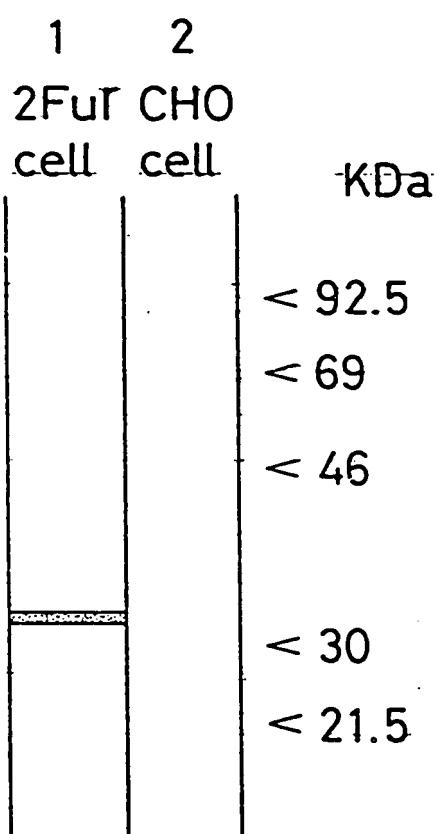
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The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. An agent comprising a monoclonal antibody which is specifically linked to a protein encoded by human chromosome 21 and which is linked to large pyramidal cells of human brain tissues.
2. The agent of claim 1 wherein the monoclonal antibody is linked to a cell nucleus protein having a molecular weight of 32 kilodaltons whose expression in brain varies dependent upon age.
3. The agent of claim 1 wherein the monoclonal antibody is prepared by immunizing mice with 2Fur cells, that is CHO cells containing long arms of human chromosome 21, fusing the resulting antibody-producing cells with mouse myeloma cells, selecting hybridomas which produce monoclonal antibodies capable of specifically recognizing a protein encoded by human chromosome 21, selecting, from these hybridomas, a hybridoma which produces a monoclonal antibody capable of recognizing a protein expressed in the human brain tissues and proliferating the hybridoma.

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FIG. 1



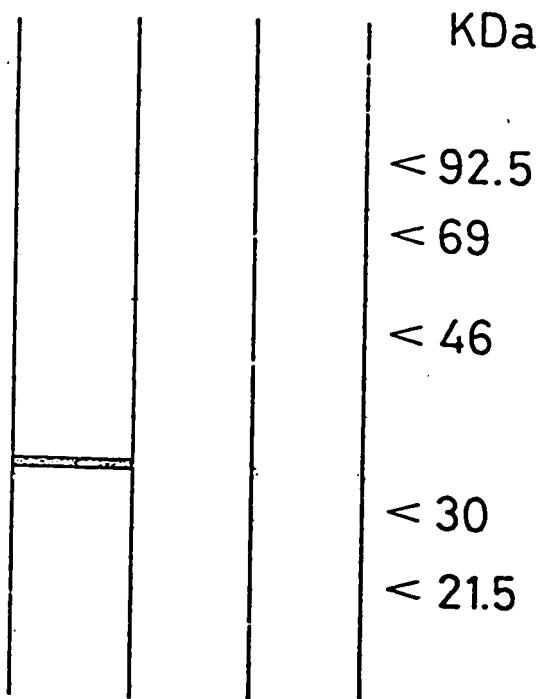
PATENT AGENTS
Swabey Ogilvy Renault

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FIG. 2

3 4 5

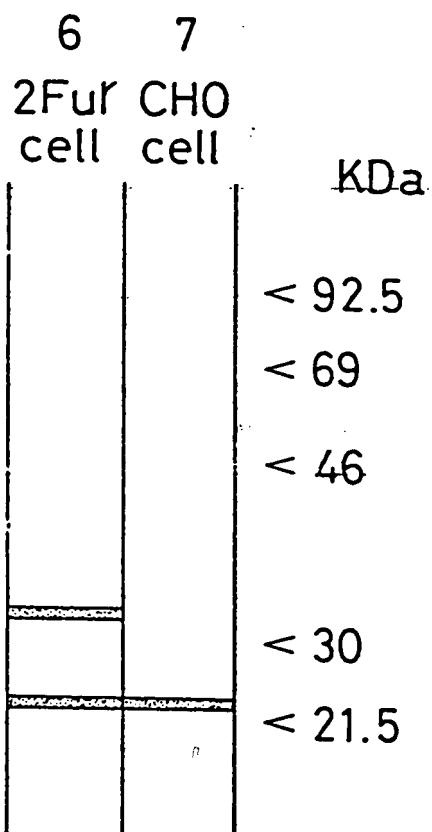
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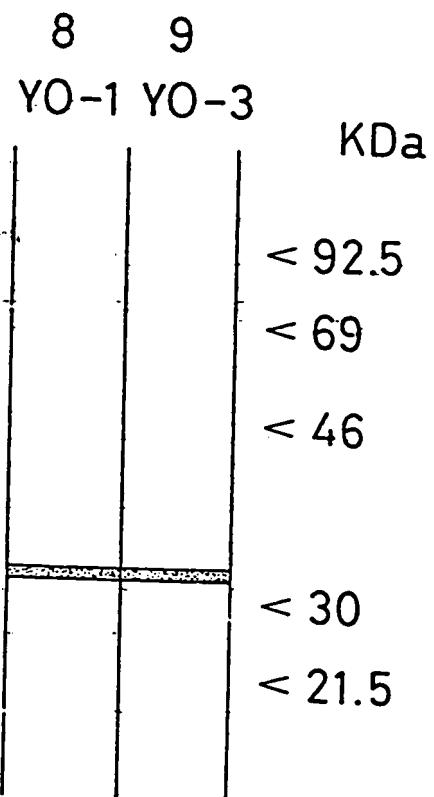
FIG. 3



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FIG. 4



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